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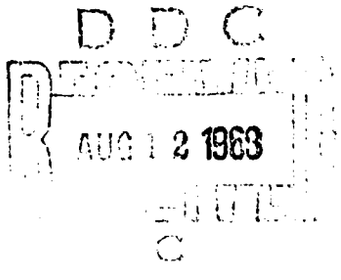

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Translated from French by Robert Butler

Biochemistry -- On the terminal groupings of the immunospecific polysaccharide of Bacillus anthracis. A paper by Laszlo Mester, Elemer Moczar and George Ivanovics, presented by Jacques Trèfouël.

(Abstract) The polysaccharide of Bacillus anthracis is shown to be a branched chain of molecules of N-acetyl-D-glucosamine, to which are joined, as terminal groupings, molecules of D-galactose in part acetyls.

An immunospecific polysaccharide has been isolated by one of us (1), (2), (3) in bacterial cells of an in vitro culture of B. anthracis on agar-agar. It is made up mostly of nearly equimolecular quantities of D-galactose, D-glucosamine and of acetic acid.

In the samples of polysaccharide studies in the present research, and isolated by the same method, but carefully avoiding all alkali action, D-galactose (40.2%) and acetic acid (12.66% acetyl) are present in a slightly higher proportion than D-glucosamine (37.8%). By paper chromatography it was shown that even a small quantity of D-xylose (2%) was present. Over and above these "-oses," the preparation contained a substance of a peptide character, connected chemically to the polysaccharide. It is made up of muramic, glutamic, aspartic and diaminopimelic acids, by alanine and by glycine.

Our previous studies (4), (5) on the structure of the polysaccharide have demonstrated the presence of N-acetyl-D-glucosamine joined in position 1:4 and of molecules of D-galactose joined in position 1:2 (or 1:4) on the one hand and in position 1:6 (or present as terminal groupings) on the other hand.

The identification by paper chromatography of the products of hydrolysis of the polysaccharide complement methyl according to the method of R. Kuhn (6) now permits us to complete our knowledge of the structure of this polysaccharide.

The exclusive presence of tetra-O-methyl-2.3.4.6. D-galactose in the hydrolysate no longer allows a choice between the molecules of D-galactose joined in position 1:6 and the molecules forming terminal groupings, but demonstrates on the one part the presence of the latter, and on the other part that the connection in position 2 (or 4) in the other half of molecules of D-galactose is sensitive to alkalines, for it disappears during methylation in an alkaline medium.

As infrared spectra have revealed the presence of O-acyl groupings in the polysaccharide, a quantitative analysis of these has been made; this gave a value corresponding approximately to a quarter of the D-galactose present. These O-acyl groupings should be made up of a surplus of acetic acid. (7)

The oxidation of the polysaccharide by periodic acid mol/10 (150 hrs at 0°C) gave 0.63 mol of formic acid in relation to the D-galactose, that is nearly a third of D-galactose molecules should have a substitution in position 2 (or 4) which would prevent the formation of formic acid during the oxidation. This result is in good agreement with the fact that nearly a fourth of the D-galactose molecules contain an O-acetyl grouping.

As to the D-glucosamine, study of the hydrolysate showed that the majority of D-glucosamine molecules, joined mostly in position 1:4, still would carry a substitution, (either a molecule of D-galactose, or another D-glucosamine molecule), which is demonstrated by the predominant presence of mono-O-methly-D-glucosamine in the hydrolysate of methyl polysaccharide. The exact identity of this derived monomethyl of glucosamine will be reported later.

According to these studies, the immunospecific polysaccharide of Bacillus anthracis is presented as a branched chain of molecules of N-acetyl-D-glucosamine, to which are joined, as terminal groupings, molecules of D-galactose, partially acetyls.

This structure is in complete agreement with the serological studies [8] and it is confirmed also by the results of the partial hydrolysis of the polysaccharide:

Partial hydrolysis of polysaccharides by 0.1 NH_2SO_4 at 80°.

Hours	D-galactose(9)	D-glucosamine(10)
0.5	5.6%	0%
1	7.8%	1.1%
3	27.0%	3.5%
16 at 100°	38.0%	7.0%

In effect by partial hydrolysis with sulfuric acid o.1 N at 80°C these are first molecules of D-galactose which become detached and even after hydrolysis of 16 hrs at 100°C only a small quantity of D-glucosamine is present in the hydrolysate.

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